

CHARACTERIZATION OF VIRULENCE POTENTIAL OF *PSEUDOMONAS AERUGINOSA* ISOLATED FROM BOVINE MEAT, FRESH FISH, AND SMOKED FISH

Comoé Koffi Donatien Benie^{1,2,*}, Adjéhi Dadié^{1,2}, Nathalie Guessenn^{2,3}, Nadège Ahou N'gbesso-Kouadio¹, N'zebo Désiré Kouame¹, David Coulibaly N'golo², Solange Aka¹, Etienne Dako⁴, Koffi Marcellin Dje¹, Mireille Dosso^{2,3}

¹Laboratory of Biotechnology and Food Microbiology (LMBM), University of Nangui-Abrogoua, 02 BP 801, Abidjan 02, Côte d'Ivoire

²Department of Bacteriology and Virology, Institut Pasteur of Côte d'Ivoire (IPCI), 01 BP 490, Abidjan 01, Côte d'Ivoire

³Faculty of Medical Sciences, University of Félix Houphouët Boigny, 01 BP V4, Abidjan 01, Côte d'Ivoire

⁴School of Food Science, Nutrition and Family Studies Faculty of Health Sciences and Community Services, University of Moncton, Moncton, NB E1A 3E9, Canada

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Pseudomonas aeruginosa owns a variability of virulence factors. These factors can increase bacterial pathogenicity and infection severity. Despite the importance of knowledge about them, these factors are not more characterized at level of strains derived from local food products. This study aimed to characterize the virulence potential of *P. aeruginosa* isolated from various animal products. Several structural and virulence genes of *P. aeruginosa* including *lasB*, *exoS*, *algD*, *plcH*, *pilB*, *exoU*, and *nan1* were detected by polymerase chain reaction (PCR) on 204 strains of *P. aeruginosa*. They were isolated from bovine meat (122), fresh fish (49), and smoked fish (33). The 16S rRNA gene was detected on 91.1% of the presumptive strains as *Pseudomonas*. The *rpoB* gene showed that 99.5% of the strains were *P. aeruginosa*. The *lasB* gene (89.2%) was the most frequently detected ($p < 0.05$). In decreasing importance order, *exoS* (86.8%), *algD* (72.1%), *plcH* (72.1%), *pilB* (40.2%), and *exoU* (2.5%) were detected. The *lasB* gene was detected in all strains of *P. aeruginosa* serogroups O11 and O16. The prevalence of *algD*, *exoS*, and *exoU* genes in these strains varied from 51.2% to 87.4%. The simultaneous determination of serogroups and virulence factors is of interest for the efficacy of surveillance of infections associated with *P. aeruginosa*.

Keywords: *Pseudomonas aeruginosa*, PCR, virulence, serogroups, bovine meat, fresh fish, smoked fish

Introduction

Pseudomonas aeruginosa is a bacterium characterized by its high genetic plasticity and potential for adapting to various environments. The species are frequently isolated from soil and water or colonizes several anatomical sites such as plants, insects, animals, and humans [1–3]. The bacterium may be involved in food poisoning and has many virulence factors [4, 5]. As consequence, *P. aeruginosa* is an opportunistic pathogen, frequently implicated in nosocomial infections [6–8].

The bacterium is found in approximately 50% to 60% of the fragile or immunocompromised people [9, 10]. It is implicated in 16% of nosocomial pneumonia, 12% in urinary tract infections, 8% in surgical infections, and 10% in bloodstream infections [11]. Otherwise, there is increasing evidence implication of the species in foodborne infections [9, 12, 13]. The pathogenicity of *P. aeruginosa* is attributed to its ability to form biofilms and to the production of numerous membrane and extracellular virulence factors [4, 5]. Membrane factors include lipopolysaccharides, flagella, adhesion factor (pili

* Corresponding author: Comoé Koffi Donatien Benie; Department of Bacteriology and Virology, Institut Pasteur of Côte d'Ivoire (IPCI), 01 BP 490 Abidjan 01, Côte d'Ivoire; Phone: +225 07 55 58 44 / +225 05 20 64 44; E-mail: dona.comoe@yahoo.fr

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type IV), and alginate, while extracellular factors are mainly exotoxins (exotoxin A), exo-proteases (elastase, staphylolysin, alkaline protease, protease IV), phospholipase C, chromophores, and exoenzymes S, T, and U [2, 9]. Virulence factors associated with the membrane are generally involved in colonization and chronic infection, while extracellular factors, extremely toxic, are associated with acute infection [9, 14]. *P. aeruginosa* can also cause lung lesions by different mechanisms. The mutation responsible for the mucoid form (muc) is associated with activation of the transcription of the alginate gene (*algD*). The formation of mucoid colonies of *P. aeruginosa* composed of alginates, involving *algD* genes, protects the bacterium from the host's immune response and from antibiotics. As lactoperoxidase (LPS), alginates are involved in the adhesion of the bacterium to the respiratory epithelium [2].

Pili are multifunctional and play a crucial role in the initiation of colonization, allowing adherence of the bacteria to host epithelial surfaces [2, 15]. Pili are also important for biofilms formation [2, 16]. These genes called *pil* are found in different regions of bacterial chromosome [17]. The expression of these genes is controlled by the two-component systems, Pil-S and Pil-R, and the alternative sigma factor RpoN.

Exoenzyme S, encoded by the *exoS* gene, is an ADP-ribosyltransferase that is secreted by the type III secretion system directly into the cytosol of epithelial cells [2, 18]. Injection of exotoxin *exoU* is the cause of rapid death (1 to 2 h) of host cells [2]. This death is characterized by loss of the integrity of the plasma membrane, which is typical of necrosis [19]. In addition to its cytotoxic activity, *exoU* induces expression of the genes of inflammation [20, 21]. *ExoU* is 100 times more cytotoxic than *exoS* according to Lee et al. [2, 19, 22]. *LasB* elastase, a zinc metalloprotease encoded by the *LasB* gene, has an elastolytic activity on lung tissue, attacks eukaryotic proteins such as collagen and elastin, and destroys the structural proteins of the cell.

In addition, the phospholipids contained in pulmonary surfactants may be hydrolyzed by two phospholipases C encoded by *plcH* and *plcN* (PLC-H and PLC-N, respectively) [2, 5]. The gene called *nan1* codes for a sialidase which is responsible for the adhesion of the respiratory tract [5, 18]. An extracellular neuraminidase is thought to play an important role in implantation of the bacterium, but the genetic basis of this process is still unknown [5, 18]. This variety of virulence factors contributes to the pathogenicity of *P. aeruginosa*. However, these virulence factors are not often studied in the strains isolated from some local food products. Those local food products and mainly products of animal origin harbor strains that may possess virulence factors and some risky zoonotic serogroups.

This study aimed to characterize the virulence factors determining the pathogenicity of *P. aeruginosa* strains isolated from animal products.

Materials and methods

P. aeruginosa isolates

The study has been carried out over a period between February 2015 and November 2015 in 5 districts of Abidjan (Côte d'Ivoire). A total of 204 isolates of *P. aeruginosa* were used in the present study. The isolates were obtained from different samples of animal products, including beef (114), fresh fish (45), and smoked fish (30). Presumptive strains of *Pseudomonas* were isolated on CFC (cetrimide, fucidine, cephaloridine; Oxoid, Code: 0559, England) base *Pseudomonas* and Cetrimide agar (Oxoid, England). *P. aeruginosa* biochemical identifications were done by API 20NE (bioMérieux, Marcy l'Etoile, France) and API database. For molecular identifications, *Pseudomonas* isolates were identified by 16S rRNA and *rpoB* gene confirmed that some of them were *P. aeruginosa*. The reference strain *P. aeruginosa* ATCC 27853 was used as quality control.

Detection of identification and virulence genes by polymerase chain reaction (PCR)

Extraction and purification of DNA

Template DNA was extracted from whole organisms by boiling [22]. Bacteria were harvested from an overnight broth culture (Biokar Diagnostics, BK015HA, France), suspended in 1 ml sterile Milli-Q water (Milli-Q™, Millipore Corporation, USA). A suspension of 200 µl was incubated at -20 °C for 15 min and boiled at 95 °C for 15 min. The suspension was immediately cooled at 4 °C for 10 min and then centrifuged at 14,000 rpm for 10 min to pellet the cell debris.

The DNA template was purified according to the method described by Zimmermann et al. [23]. The purity and DNA concentration of the extract were determined by spectrophotometer (Eppendorf BioPhotometer plus, USA).

Amplification of 16S rRNA, *rpoB*, *algD*, *PilB*, *lasB*, *nan1*, *plcH*, *exoS*, and *exoU* genes

Single PCR was used to detect 16S RNA and *rpoB* gene. PCR mixtures with a final volume of 25 µl consisted of 16 µl sterile Milli-Q water (Milli-Q™, Millipore Corporation, USA), 5 µl 5XTP, 1.5 µl MgCl₂ (2 mM), 0.2 µl dNTPs (10 mM), 0.1 µl each primer (20 mM) (Integral DNA Technology, France), 0.1 µl Go tag polymerase (Promega Corporation, Madison, WI 53711-5399, USA), and 2 µl template DNA.

Multiplex PCRs were used to detect *algD*, *PilB*, *lasB*, *nan1*, *plcH*, *exoS*, and *exoU*. PCR mixtures with a final volume of 25 µl consisted of 15.8 µl sterile Milli-Q water (Milli-Q™, Millipore Corporation, USA), 5 µl

Table 1. Primers used for amplification of identification genes in single PCR

Primers	Target gene	Sequence (5'-3')	Product size (bp)	Amplification program	Annealing temperature (°C)	Source
16S-F 16S-R	16S rRNA	AGAGTTTGATCCTGGCTCAG CTACGGCTACCTTGTTCAG	≈1351	94 °C, 2 min 5 × [94 °C, 45 s; 55 °C, 1 min; 72 °C, 2 min] 35 × [92 °C, 45 s; 60 °C, 45 s; 72 °C, 2 min] 72 °C, 2 min; 4 °C...	55 60	[25]
rpoB-F rpoB-R	<i>rpoB</i>	CAGTTCAIAGGACCAGAACACCCG ACGCTGGTTGATGCAGGTGTTTC	≈759	94 °C, 3 min 30 × [94 °C, 1 min; 58 °C, 1 min; 72 °C, 2 min] 72 °C, 2 min; 4 °C...	58	[27]

PCR: polymerase chain reaction; F: forward; R: reverse

Table 2. Primers used for amplification of virulence genes in multiplex PCR

Primers	Target gene	Sequence (5'-3')	Product size (bp)	Amplification program	Annealing temperature (°C)	Source
pilB-F pilB-R	<i>pilB</i>	ATG AAC GAC AGC ATC CAA CT GGG TGT TGA CGC GAA AGT CGA T	826			[29]
LasB-F LasB-R	<i>LasB</i>	GGA ATG AAC GAG GCG TTC TC GGT CCA GTA GTA GCG GTT GG	300	94 °C, 5 min 35 × [94 °C, 35 s; 60 °C, 1 min; 72 °C, 1 min] 72 °C, 7 min; 4 °C...	60	[28]
ExoS-F ExoS-R	<i>exoS</i>	CTT GAA GGG ACT CGA CAA GG TTC AGG TCC GCG TAG TGA AT	504			[28]
algD-F algD-R	<i>algD</i>	ATG CGA ATC AGC ATC TTT GGT CTA CCA GCA GAT GCC CTC GGC	1310	94 °C, 5 min 35 × [94 °C, 35 s; 61 °C, 1 min; 72 °C, 1 min] 72 °C, 7 min; 4 °C...	62 60	[28] [28]
plcH-F plcH-R	<i>plcH</i>	GAA GCC ATG GGC TAC TTC AA AGA GTG ACG AGG AGC GGTAG	307			[28]
nanI-F nanI-R	<i>nanI</i>	ATG AAT ACT TAT TTT GAT AT CTA AAT CCA TGC TCT GAC CC	1317	94 °C, 5 min 35 × [94 °C, 35 s; 57 °C, 1 min; 72 °C, 1 min] 72 °C, 7 min; 4 °C...	53	[29]
exoU-F exoU-R	<i>exoU</i>	GGG AAT ACT TTC CGG GAA GTT CGA TCT CGC TGC TAA TGT GTT	428			[29]

PCR: polymerase chain reaction; F: forward; R: reverse. *AlgD*, GDP-mannose 6-dehydrogenase AlgD (alginate)-encoding gene; *pilB*, type IV fimbrial biogenesis protein PilB-encoding gene; *nanI*, neuramidase-encoding gene; *plcH*, hemolytic phospholipase C precursor-encoding gene; *exoS*, elastase LasB-encoding gene; *exoU*, exoenzyme U-encoding gene

5XTP, 1.5 μ l MgCl₂ (2 mM), 0.2 μ l dNTPs (10 mM), 0.1 μ l each primer (20 mM) (Integral DNA Technology, France), 0.1 μ l Go tag polymerase (Promega Corporation, Madison, WI 53711-5399, USA), and 2 μ l DNA template. A quantity of 2 μ l of sterile Milli-Q water was used for negative control, and DNA of ATCC reference strain 27853 was used for positive control.

The oligonucleotide primers used in this study and the amplification program are listed in *Tables 1* and *2*. Each PCR was performed using thermocycleur type T3000 Thermocycler, block type standard 3a (Biometra, Germany). The amplified DNA was separated by gel electrophoresis with 2% agarose containing 0.5 μ g/ml with ethidium bromide for 30 min at 130 V, visualized under UV transillumination, and photographed (Molecular Imager Gel Doc™ XR+, Bio-Rad). A molecular weight marker (TriDye™, 100 bp or 1-kb DNA Ladder, Biolabs) was used. Amplified genes were identified on the basis of fragment size shown in *Tables 1* and *2*.

Serotyping of *P. aeruginosa* isolates

The O-serotypes were determined by a slide agglutination test using four pools (OMA, OMC, OME, and OMF) and 20 monovalent antisera, O1 to O20 (Sanofi Diagnostics Pasteur), according to the manufacturer's recommendations.

Statistical analysis

The statistical analysis was carried out on the software Statistical Package for the Social Sciences (SPSS) 20.0 (IBM SPSS, Chicago, IL, United States of America). The Student's *t* test, Mann–Whitney *U* test, Spearman's correlation analysis, and multiple regression analysis were performed. Statistical significance was set at $p < 0.05$.

Results

P. aeruginosa strains detected by 16S rRNA and rpoB genes

Out of 225 presumptive isolates of *Pseudomonas*, 205 (91.1%) *Pseudomonas* were identified by 16S rRNA (*Fig. 1*; *Table 3*) and 204 (99.5%) strains were confirmed by the *rpoB* gene (*Fig. 2*; *Table 3*) as *P. aeruginosa*.

Virulence genes of *P. aeruginosa*

Out of seven genes researched, six virulence genes of *P. aeruginosa* were detected in some animal samples (line 5: *lasB*, *exoS*, *algD*, *plcH*, *pilB*, and *exoU*; *Figs. 3–5*). Other samples have fewer than six virulence factors (lines 2–4 and 6–13; *Figs. 3–5*).

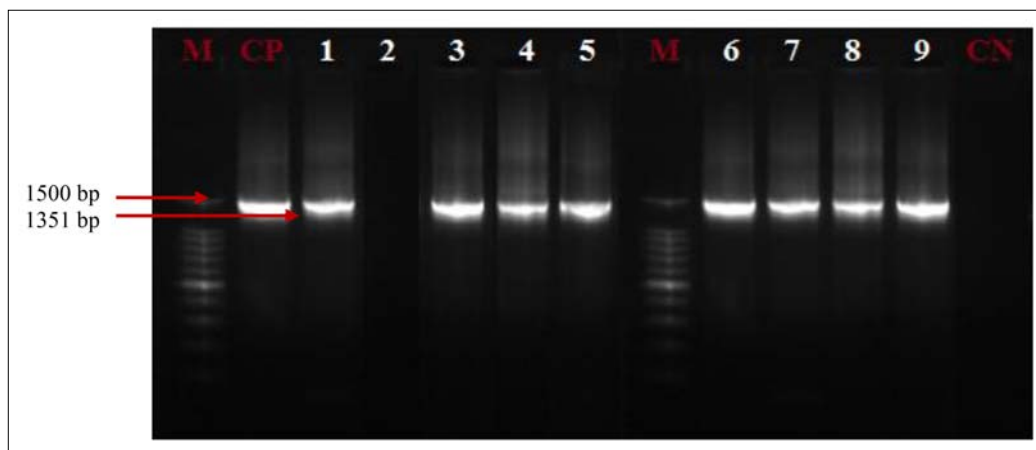


Fig. 1. 16S rRNA profiles of *Pseudomonas* isolates. Lanes 1, 3–9: Presence of *Pseudomonas* in analyzed products; lane 2: absence of *Pseudomonas* in analyzed products; CP: positive control (*Pseudomonas aeruginosa* ATCC 27853); CN: negative control; M: marker gene ruler, 100 bp (Bench Top, 100-bp DNA Ladder, Promega Corporation, USA)

Table 3. Frequency of strains confirmed by the 16S rRNA and *rpoB* genes

Genes	Number of isolates presumptive <i>Pseudomonas</i> N = 225		
	Confirmed species	Effective	Percentage
16S rRNA	<i>Pseudomonas</i> SP	205	91.1
<i>rpoB</i>	<i>Pseudomonas aeruginosa</i>	204	99.5

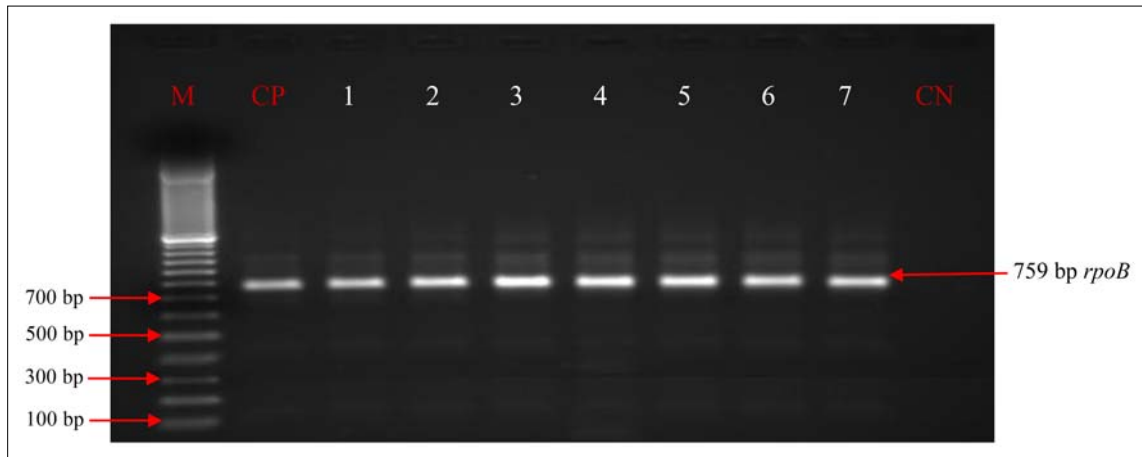


Fig. 2. RpoB profiles of *Pseudomonas aeruginosa* isolates. Lanes 1–7: Presence of *Pseudomonas aeruginosa* in analyzed products; CP: positive control (*Pseudomonas aeruginosa* ATCC 27853); CN: negative control; M: marker gene ruler, 100 bp (Bench Top, 100-bp DNA Ladder, Promega Corporation, USA)

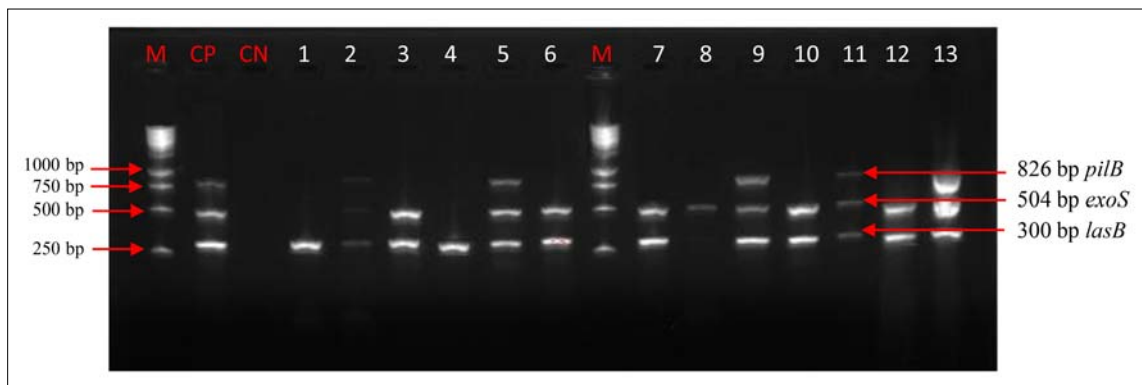


Fig. 3. Electrophoretic profile of amplification products of the virulence genes *pilB*, *exoS*, and *lasB* in analyzed products. Virulence genes were present in analyzed products. Lane 1: *lasB*; lane 2: *pilB*, *exoS*, and *lasB*; lane 3: *exoS* and *lasB*; lane 4: *lasB*; lane 5: *pilB*, *exoS*, and *lasB*; lane 6: *exoS* and *lasB*; lane 7: *exoS* and *lasB*; lane 8: *exoS* and *lasB*; lane 9: *pilB*, *exoS*, and *lasB*; lane 10: *exoS* and *lasB*; lane 11: *pilB*, *exoS*, and *lasB*; lane 12: *exoS* and *lasB*; lane 13: *pilB*, *exoS*, and *lasB*; M: marker gene ruler, 250 bp (Bench Top, 1-kb DNA Ladder, Promega Corporation, USA); *pilB*, type IV fimbrial biogenesis protein PilB-encoding gene; *lasB*, elastase LasB-encoding gene; *exoS*, exoenzyme S-encoding gene

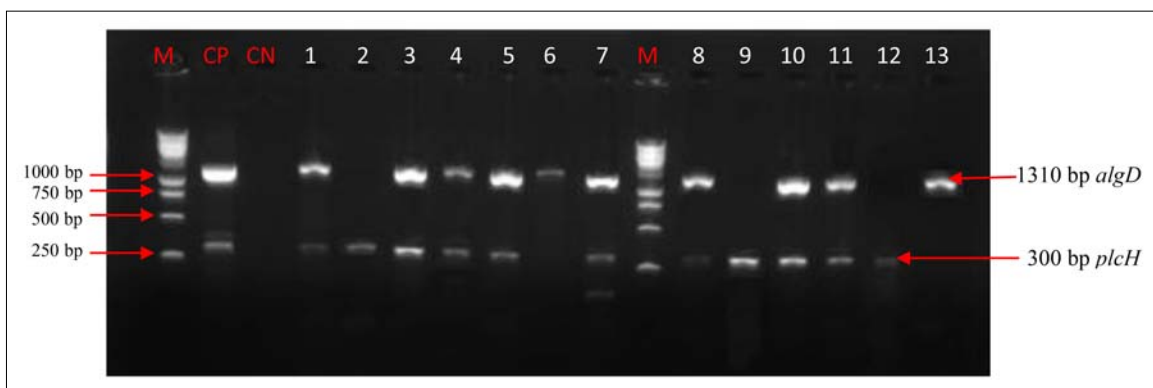


Fig. 4. Electrophoretic profile of amplification products of the virulence genes *algD* and *plcH* in analyzed products. Virulence genes were present in analyzed products. Lane 1: *algD* and *plcH*; lane 2: *plcH*; lane 3: *algD* and *plcH*; lane 4: *algD* and *plcH*; lane 5: *algD* and *plcH*; lane 6: *algD*; lane 7: *algD* and *plcH*; lane 8: *algD* and *plcH*; lane 9: *plcH*; lane 10: *algD* and *plcH*; lane 11: *algD* and *plcH*; lane 12: *plcH*; lane 13: *algD*; M: marker gene ruler, 250 bp (Bench Top, 1-kb DNA Ladder, Promega Corporation, USA); *algD*, GDP-mannose 6-dehydrogenase AlgD (alginate)-encoding gene; *plcH*, hemolytic phospholipase C precursor-encoding gene

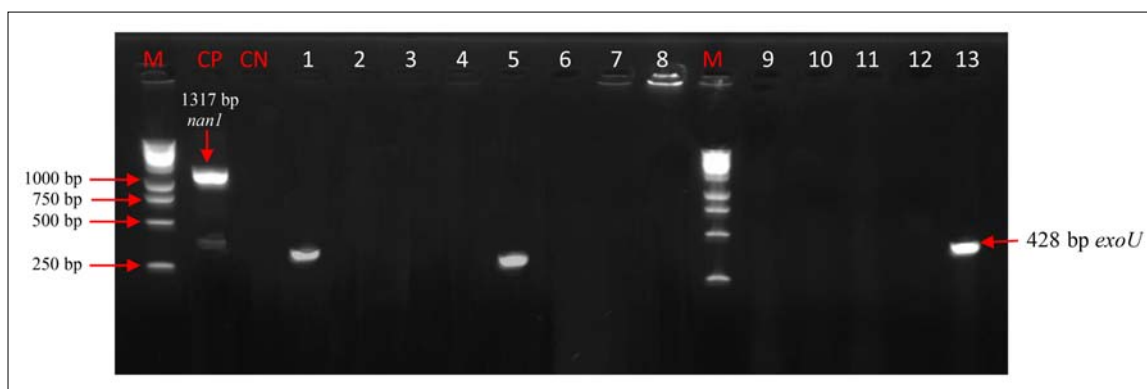


Fig. 5. Electrophoretic profile of amplification products of the virulence gene *exoU* and *nanI* in analyzed products. Virulence genes were present in analyzed products. Lanes 1, 5, 13: *exoU*; Virulence genes were absent in analyzed products; lanes 2–4, 6–12; M: marker gene ruler, 250 bp (Bench Top, 1-kb DNA Ladder, Promega Corporation, USA); *algD*, GDP-mannose 6-dehydrogenase AlgD (alginate)-encoding gene; *plcH*, hemolytic phospholipase C precursor-encoding gene. M: marker gene ruler, 250 bp (Bench Top, 1-kb DNA Ladder, Promega Corporation, USA); *exoU*, exoenzyme U-encoding gene

Table 4. Prevalence of *P. aeruginosa* virulence genes isolated from animal products ($n = 204$)

Virulence genes	Prevalence of <i>Pseudomonas aeruginosa</i> virulence genes isolated from animal products							
	Bovine meat ($n = 122$)		Fresh fish ($n = 49$)		Smoked fish ($n = 33$)		Total ($n = 204$)	
	Effective (N)	Prevalence (%)	Effective (N)	Prevalence (%)	Effective (N)	Prevalence (%)	Effective (N)	Prevalence (%)
<i>lasB</i>	118	96.7	35	71.4	29	87.9	182	89.2
<i>exoS</i>	118	96.7	33	67.3	26	78.8	177	86.8
<i>algD</i>	91	74.5	38	77.5	18	54.5	147	72.1
<i>plcH</i>	88	72.1	37	75.5	22	66.7	147	72.1
<i>pilB</i>	52	42.6	19	38.8	11	33.3	82	40.2
<i>exoU</i>	5	4.1	0	0.0	0	0.0	5	2.5
<i>nanI</i>	0	0.0	0	0.0	0	0.0	0	0.0

AlgD, GDP-mannose 6-dehydrogenase AlgD (alginate)-encoding gene; *pilB*, type IV fimbrial biogenesis protein PilB-encoding gene; *nanI*, neuraminidase-encoding gene; *plcH*, hemolytic phospholipase C precursor-encoding gene; *lasB*, elastase LasB-encoding gene; *exoS*, exoenzyme S-encoding gene; *exoU*, exoenzyme U-encoding gene

Prevalence of virulence genes

Table 4 showed the prevalence of genes detected out of 204 strains of *P. aeruginosa*. According to the table, *lasB* gene with 89.2% has been most detected following by *exoS* (86.8%). Genes *algD* and *plcH* had the same prevalence (72.1%). The prevalence of *pilB* and *exoU* genes was, respectively, 40.2% and 2.5%. The *nanI* gene had not been detected during this study.

The prevalence of virulence genes varied from 0% to 96.7%, from 0% to 87.9%, and from 0% to 77.5%, respectively, in bovine meat, smoked fish, and fresh fish (Table 4). The prevalence of *lasB* and *exoS* genes was higher in bovine meat (96.7% and 96.7%), followed by smoked fish (87.9% and 78.8%) and fresh fish (71, 4%, and 67.3%) (Fig. 6). That of *algD* and *plcH* gene was respectively higher in fresh fish (77.5% and 75.5%), followed by bovine meat (74.5% and 72.1%) and smoked fish (54.5% and 66.7%) (Fig. 6). The prevalence of the *pilB* gene was less than 50% in all animal products analyzed (Fig. 6). The *exoU* gene was detected only in strains from bovine meat.

Table 5. Serogroups of *Pseudomonas aeruginosa*

Serogroups	Number of strains ($n = 204$)	Prevalence (%)
O11	52	25.5
O5	43	21.1
O16	42	20.6
NS	10	4.9
O7	10	4.9
O8	8	3.9
O9	8	3.9
O15	7	3.4
O1	6	2.9
O10	6	2.9
O12	6	2.9
O2	3	1.5
O4	3	1.5

NS: not serotypeable; O: serogroups

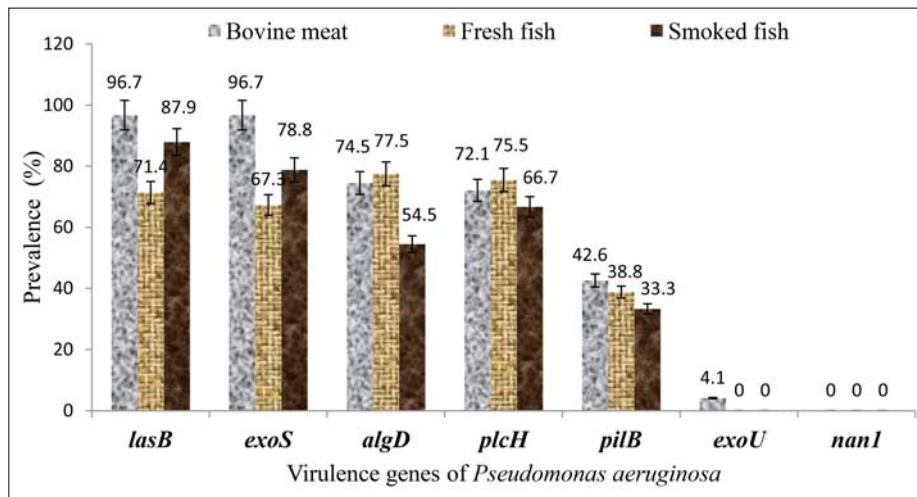


Fig. 6. Virulence genes according to the origin of the strain. *AlgD*, GDP-mannose 6-dehydrogenase AlgD (alginate)-encoding gene; *pilB*, type IV fimbrial biogenesis protein PilB-encoding gene; *nanI*, neuraminidase-encoding gene; *plcH*, hemolytic phospholipase C precursor-encoding gene; *lasB*, elastase LasB-encoding gene; *exoS*, exoenzyme S-encoding gene; *exoU*, exoenzyme U-encoding gene

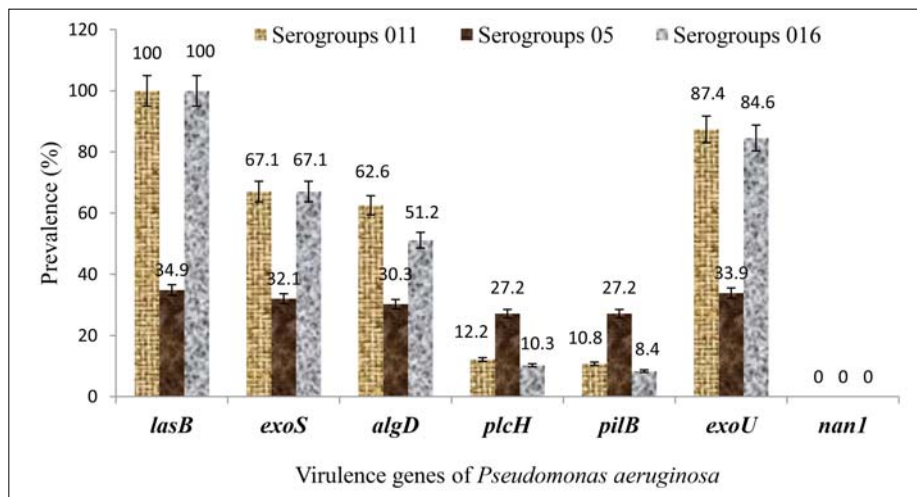


Fig. 7. Virulence genes and serogroups of *Pseudomonas aeruginosa*. *AlgD*, GDP-mannose 6-dehydrogenase AlgD (alginate)-encoding gene; *pilB*, type IV fimbrial biogenesis protein PilB-encoding gene; *nanI*, neuraminidase-encoding gene; *plcH*, hemolytic phospholipase C precursor-encoding gene; *lasB*, elastase LasB-encoding gene; *exoS*, exoenzyme S-encoding gene; *exoU*, exoenzyme U-encoding gene

Diversity of P. aeruginosa serogroups

The mostly isolated serogroups were O11 with 25.5%, followed by serogroups O5 (21.1%) and O16 (20.6%). According to Table 5, non-serotyping strains and serogroups O7, O8, O9, O15, O1, O10, O12, O2, and O4 were less than 5%.

Prevalence of the virulence genes of P. aeruginosa according to serogroups

Elastase-producing strains of *P. aeruginosa* (*lasB*) belonging to serogroups O11 and O16 had a prevalence of 100% (Fig. 7). However, the same strains of serogroups O11 and O16 harbored the alginate (*algD*), exoenzyme

(*exoS*), and (*exoU*) with a prevalence ranging from 51.2% to 87.4%. Elsewhere, genes (*plcH*) and (*pilB*) had been detected from 8.4% to 12.2% (Fig. 7). *P. aeruginosa* strains belonging to O5 serogroups had a prevalence ranging from 27.2% to 34.9% for strains with virulence factors. No strain of the three serogroups produced neuraminidase 1.

Distribution of P. aeruginosa virulence genes

According to Fig. 8, virulence factors have been distributed at 99.7% on the axes F1 and F2. The F1 axis represents the F1 axis represents 97.1%, and the F2 axis, 2.6% (Fig. 8). All the variables bovine meat, fresh fish, and smoked fish are positively represented on axis 1. This axis contrasts the *lasB*, *exoS*,

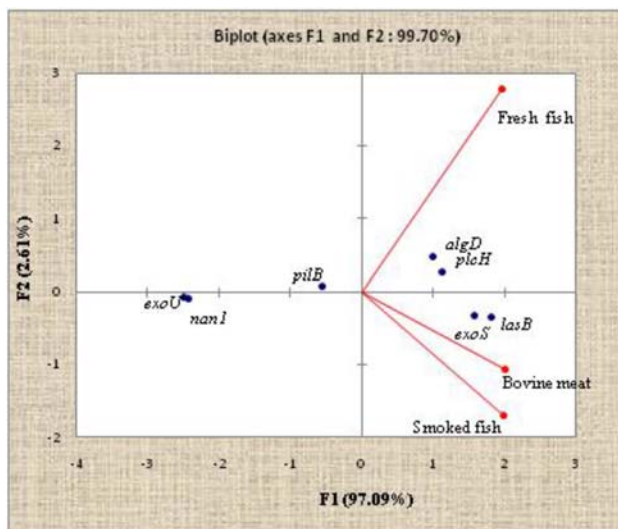


Fig. 8. Distribution of *Pseudomonas aeruginosa* virulence genes isolated from animal products. The axes F1 and F2 of the ACP account for 99.7% of the inertia. The F1 axis represents 97.09% and the F2 axis 2.61%. *AlgD*, GDP-mannose 6-dehydrogenase AlgD (alginate)-encoding gene; *pilB*, type IV fimbrial biogenesis protein PilB-encoding gene; *nanI*, neuraminidase-encoding gene; *plcH*, hemolytic phospholipase C precursor-encoding gene; *lasB*, elastase LasB-encoding gene; *exoS*, exoenzyme S-encoding gene; *exoU*, exoenzyme U-encoding gene

algD, and *plcH* genes that are positively correlated with the *pilB*, *exoU*, and *nanI* genes which correlation is negative. Thus, the *lasB*, *exoS*, *algD* and *plcH* genes are the most found in the three food matrices (bovine meat, fresh fish, and smoked fish).

Discussion

16S rRNA gene analysis revealed that 91.1% of the analyzed food products were positive for the genus *Pseudomonas*, whereas in 99.5% of the *Pseudomonas*-positive samples, the *rpoB* gene could be detected, indicating contamination of the products by *P. aeruginosa*. This result shows that the resolving power of this marker is higher than that of 16S rRNA [25]. This result also highlights the strong discriminating power of the identification method using *rpoB* gene and confirms the heterogeneity of the *P. aeruginosa* observed by many authors [25, 29]. This high molecular identification rate showed that genomic studies are needed to confirm the exact taxonomic position of *P. aeruginosa*. The performance of the *rpoB* gene on the detection of *P. aeruginosa* strains could also be explained by the fact that the discrimination between species very close to *Pseudomonas* is obtained by the analysis of the *rpoB* gene [25, 30].

The results also showed that the prevalence of virulence genes of *P. aeruginosa* predominantly detected was 89.2% for *lasB* and 86.8% for *exoS*, followed by 72.1% for *algD* and 72.1% for *plcH*. The high prevalence of elastase (89.2%) encoding the *lasB* (zinc metalloprotease)

gene and secreted by type II secretion system indicates that this protease can be important for the pathogenesis of *P. aeruginosa* isolated from animal products cleaving elastin and collagen [2, 5].

Indeed, this enzyme is at the origin of a destruction of the junctions between the epithelial cells [2, 5]. This protease increases the production of IL-8 and reduces the innate immune response by cleaving proteins from surfactant, SP-A and SP-D, and protease receptors [5]. Elastase B is also able of inactivating other proteins such as IgA, IgG, and complement compounds, thus, modulating the immune response. It also inhibits the repair of injured epithelial cells by altering cell mobility [2–4].

The high prevalence of *algD* gene could be at the origin of the conversion of *P. aeruginosa* strains to a mucoid phenotype overproducing alginates [31]. This alginate prevalence indicates that isolated strains of animal products are involved in the formation of biofilms as alginates have been widely regarded as the major exopolysaccharides of the biofilm matrix [31, 32]. The overproduction of alginates protects *P. aeruginosa* from antibiotics and also alleviates the immune response by inhibiting complement activation, reducing polymorphonuclear chemotaxis and decreasing phagocytosis [31]. The expression of *algD* and *lasB* genes could be correlated with appearance of a mucoid phenotype. Also, alginate and elastase production could be characteristic of installation of a chronic infection in consumers of animal products [32]. These same high prevalences of these virulence genes were obtained by Mitov et al. [4].

The prevalences for the *plcH* and *exoS* genes were 72.1% and 86.8%, respectively. These prevalences showed that strains isolated were able of secreting hemolytic exoenzymes and phospholipase C, and thus, strains could be involved in pulmonary infections [33, 34]. The *exoS* was responsible for tissue destruction; it is implicated to pulmonary infection and can be important for bacterial dissemination [4]. This prevalence was 40.2%, 2.5%, and 0.0%, respectively, for the *pilB*, *exoU*, and *nanI* gene. The prevalence of the *pilB* gene which codes for the formation of pili indicates that the strains are also involved in the twitching motility which allows the displacement on a solid surface, thanks to the extension and then the retraction of the pili and the mobility of the “swarming” type [16]. This prevalence shows that strains isolated from animal products could be involved in the formation of biofilm and the initiation of infections by bacteriophages whose pili is considered as a receptor.

The relatively low prevalence for exoenzyme-U could be explained by the fact that 90% of *P. aeruginosa* strains producing *exoU* are associated with serious infections, and therefore, the strains of animal origin are not extremely associated with severe infections [4, 19]. Among type III secretion proteins, *exoU* is the most cytotoxic gene. The *exoU* secretion is a marker of invasive isolates of *P. aeruginosa* [5, 19].

These results indicate that some of virulence factors assist bacterial establishment and colonization on the sur-

face of the host, while others expedite invasion of numerous tissue. Some factors such as flagella, fimbriae, surface polysaccharides, and type IV pili are involved in bacterial colonization. All these elements are considered essential for attachment mechanism. *P. aeruginosa* has the ability to invade tissue. *P. aeruginosa* also produces toxins and enzymes that disrupt carnal barriers by disrupting cell membranes despite the immune system of the host.

In addition, the results of this study showed that all elastase-producing strains of *P. aeruginosa* (*lasB*) belonged to serogroups O11 and O16 with a prevalence of 100%. Recently, Le-Berre et al. showed that the O11 serogroups, elastase production, and TTSS were associated with increased lung injury in a murine model of pneumonia [35]. They found that serogroups O11 strains were significantly more virulent than nontypable strains and serotype O6.

These same strains of serogroups O11 and O16 had prevalence between 51.2% and 87.4% for the alginate (*algD*), exoenzyme (*exoS*), and (*exoU*) producing strains. *P. aeruginosa* strains belonging to O5 serogroups had a prevalence ranging from 27.2% to 34.9% for all the strains with virulence factors in this study. Neuraminidase was not produced by any strains of the three serogroups produced.

All these results could indicate that strains of O11 and O16 serogroups are more associated with virulence factors than strains belonging to O5 serogroups [36, 37].

The results of this study showed that *lasB*, *exoS*, *algD*, and *plcH* genes are the most frequently found in the three food matrices. *P. aeruginosa* strains isolated from animal products are able to harbor genes of resistance and virulence belonging to particular serogroups.

Conclusion

The study showed that the *rpoB* gene confirms the identification of *P. aeruginosa*. It also revealed that strains of *P. aeruginosa* isolated from animal products harbor distinct virulence genes. The *lasB*, *exoS*, *algD*, and *plcH* genes are the most found in the three food matrices. The study also indicated that strains of serogroups O11 and O16 were more associated with the virulence genes of *P. aeruginosa* isolated from animal products.

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Conflict of interest

The authors declare that there is no conflict of interest.

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